What do the results mean?

Estimating disease prevalence in the 50 US states may sound a bit dull to some readers, but quantifying such burden of disease is a crucial step in planning appropriate services, especially if such data can be coupled with disease severity and health-care usage (Chren and Weinstock, 2004). The data thus provide the most basic of building blocks for a Needs Assessment of National and State dermatology services for young people (Schofield *et al.*, 2009). Second, the variation in disease prevalence with factors such as geographical location, ethnic group, and educational status, if true, signals that the environment is critical for determining disease expression in eczema. Although genetic factors such as filaggrin gene mutations help to explain some variation in eczema (Rodríguez *et al.*, 2009), it is likely that interaction with environmental factors plays a critical role given the associations shown in this study and the increasing prevalence of disease over the past 20 years (Williams *et al.*, 2008). The study findings do not mean that a young, black, well-educated, English-speaking resident of a city on the East Coast of the United States will necessarily get eczema, because these associations relate to aggregated risks and because they refer to attributes that point to more specific exposures such as diet, hygiene, and health behaviors. The challenge now is to identify the environmental factors that are amenable to public health manipulation in the hope that this knowledge will bring us one step closer to eczema prevention (Mar and Marks, 2000).

CONFLICT OF INTEREST

The author has worked with the International Study of Asthma and Allergies (http://isaac.auckland.ac.nz) for the past 14 years.

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Urocanic Acid in the Skin: A Mixed Blessing?

Neil K. Gibbs[1] and Mary Norval[2]

Located in the stratum corneum, urocanic acid is a major epidermal chromophore for UVR. This simple molecule has attracted a great deal of research interest over the past half century, initially as a putative "natural sunscreen" and later as a mediator of photoimmunosuppression with a consequent role in photocarcinogenesis. For the first time, Barresi and colleagues provide robust evidence for the photoprotective role of endogenous urocanic acid and reopen the debate on the relative "beneficial" and "detrimental" properties of this molecule.

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Urocanic acid (UCA) was originally isolated from the urine of a dog by Jaffé in 1874, but the presence of epidermal UCA was only confirmed in the 1950s. *Trans*-UCA is produced in the mammalian stratum corneum by the action of L-histidine ammonia lyase (histidase; *Hal*) on histidine. The major source of histidine in this skin compartment is filaggrin (formerly called histidine-rich protein) and reduced production of filaggrin results in lower levels of UCA. Urocanase, the enzyme that catabolizes UCA in the liver, is absent in the skin, which allows accumulation of UCA up to 0.5% of the dry weight of the epidermis. In humans, epidermal UCA levels range from 4 to 34 nM/cm^2 and do not correlate with any parameter so far tested including age, sex, pigmentation, skin phototype, and minimal erythema dose (see, for example, de Fine Olivarius *et al.*, 1997). UCA has been proposed as the major acid–base regulator in the epidermis, although recent results demonstrate that the filaggrin–histidase–UCA cascade

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is not essential for the acidification of the surface of the stratum corneum (Fluhr *et al.,* 2010).

> Beneficial photo protection by exogenous urocanic acid contrasts with its detrimental photoimmuno-suppressive effects.

It was also established more than 50 years ago that UCA is a major epidermal chromophore for UVR, which convinced investigators that the molecule existed in the stratum corneum as a "natural sunscreen" (Tabachnick, 1957). As the photochemical properties and importance of DNA became understood, the fact that the UCA absorption spectrum partially overlapped the DNA absorption spectrum added credence to this idea and led the cosmetics industry to include UCA in their skin products. This remained the situation for the following quarter of a century, which saw significant advances in UCA photochemistry; it became clear that *trans*-UCA could be photoisomerized to *cis*-UCA on UVR exposure and this conversion was considered a "safe" way to disperse photon energy. The photoisomerization reaction was reversible and both UCA isomers had very similar absorption properties. Such findings enhanced UCA's reputation as an ideal natural sunscreen.

This "understanding" of the role of epidermal UCA was shattered with the seminal finding that UCA was a chromophore for, and *cis*-UCA a mediator of, the immunosuppressive effects of UVR in the skin (de Fabo and Noonan, 1983). Subsequent work by other groups confirmed the original finding, which prompted de Fabo and Noonan to submit a citizens' petition in 1991 requesting the US Food and Drug Administration to find that "urocanic acid is a deleterious substance and may render any cosmetic product containing it injurious." A review by the US Cosmetic Ingredient Review Expert Panel and the gradual removal of UCA from cosmetic products followed. From 1983 to date, research on UCA, with a few exceptions, has been dedicated to understanding its role in photoimmunosuppression and how this may increase photocarcinogenic risk. As a consequence, the putative photoprotective role of UCA has not been considered a topic of much relevance or interest until now.

Early photoprotection experiments in guinea pigs, and more recently in humans, have demonstrated that photoprotection offered by topical application of UCA is limited and approximately equivalent to a sunscreen with a sun protection factor (SPF) of 1.5. However, in these types of studies, exogenous UCA was administered above the UCA already existing in the stratum corneum, so the efficacy of UCA as an endogenous photoprotectant *per se* was not assessed. Barresi and colleagues (2011, this issue) elegantly address this question using mice that are histidase-deficient because of a mutation in the *Hal* gene. These histidinemic animals have less than 10-fold the concentration of UCA in their stratum corneum than wild-type mice. Although a strain of histidase-deficient mice was available in the past (they were mentioned in de Fabo and Noonan, 1983), these animals were sickly and did not breed well. Barresi and colleagues' study used animals resulting from a backcross between these original "Peruvian" mice and C57BL/6 mice, which has provided a healthier histidinemic strain. The authors demonstrate that after broadband UVB irradiation of 250 mJ cm^{-2} (which is likely to represent an erythemal dose), the levels of DNA damage (thymine dimers; T^T) and markers of apoptosis (caspase-3 and TUNEL) were about 40% higher in the epidermis of the histidinemic mice than in wild-type animals. The photosensitivity of the histidinemic mice to T^T induction could be abrogated by the topical application of a high concentration of UCA.

Thus, for the first time, a photoprotective role for endogenous UCA has been demonstrated in mammalian skin. Quantitatively, the protection is similar to that afforded by topical UCA applied exogenously in the mouse and human studies conducted—an SPF of about 1.5—which at first glance may seem small. However, unlike the vagaries of sunscreen application, endogenous UCA is a constitutive photoprotectant that should reduce some major deleterious UVR-induced effects by 33%.

The action spectrum for UVR-induced erythema in mammalian skin closely resembles that for T^T induction and erythemal responses are exaggerated if repair of these lesions is deficient, such as in certain xeroderma pigmentosum complementation groups. It would therefore be expected that the protection offered by endogenous UCA against T^T, as demonstrated by Barresi and colleagues (2011), would translate to protection against erythema. Interestingly, the authors did not note any gross differences in skin pathology between the histidinemic and wild-type mice 24 hours after irradiation. One study examining the relationship between cutaneous UCA levels and erythemal photosensitivity in humans found that, despite a 10-fold variation in UCA concentration, there was no correlation between these two parameters (de Fine Olivarius *et al.*, 1997). This is surprising because a strong inverse relationship would be expected between the concentration of UCA and UVR transmission if UCA was acting purely as a chemical sunscreen in the stratum corneum, and it suggests that UCA is acting in a more complex manner. Indeed, it has been variously reported that UCA can also photosensitize DNA damage such as 6-4 photoproducts *in vivo* or cyclobutane adducts *in vitro*. Recent evidence suggests that treatment of cells with *cis*-UCA can produce oxidative DNA damage (8-oxo-deoxyguanosine) via the induction of intracellular reactive oxygen species (ROS; Sreevidya *et al.*, 2010). In keratinocyte cultures *cis*-UCA induced genes associated with apoptosis, cell growth arrest, and oxidative stress (Kaneko *et al.*, 2008). The comparative contributions of these opposing mechanisms to the erythemal response merit further investigation.

The implications of the findings of Barresi and coworkers (2011) for photoimmunosuppression are intriguing. Experiments in mice reveal that removing or enhancing the repair of UVR-induced T^T, using photolyase or T4 endonuclease, reduces photoimmunosuppression, thus suggesting a protective role for endogenous UCA in this pathway. In contrast, *cis*-UCA is an

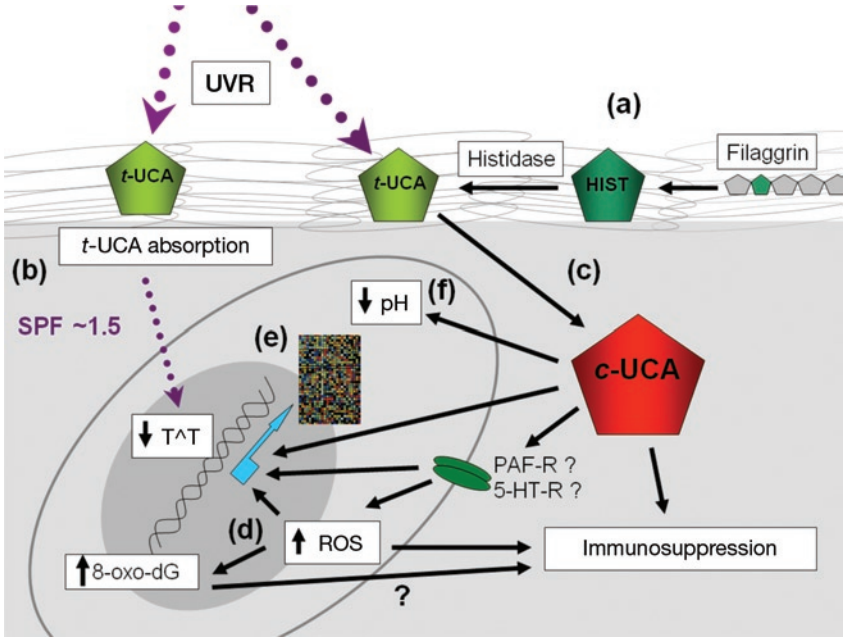

Figure 1. Putative "beneficial" and "detrimental" effects of urocanic acid (UCA). (**a**) *trans*-UCA is formed in the stratum corneum from histidine (HIST) released from filaggrin; (**b**) exogenous *trans*-UCA absorbs UVR and protects against thymine dimer formation in keratinocytes; (**c**) *cis*-UCA is formed by photoisomerization of *trans*-UCA; (**d**) acting through membrane receptors, *cis*-UCA induces intracellular reactive oxygen species (ROS) and hence oxidative DNA damage (8-oxo-dG); (**e**) *cis*-UCA initiates translation of genes associated with apoptosis and immunosuppression through direct signaling and intracellular ROS formation; and (**f**) *cis*-UCA provides proapoptotic intracellular acidification in tumor cells. SPF, sun protection factor, T^T, thymine dimers.

immunosuppressant. A report that intracellular ROS may be induced by *cis*-UCA (Sreevidya *et al.*, 2010) may explain what initially seemed a strange finding that an antioxidant (*N*-acetylcysteine) could reduce the immunosuppressive effects of topically applied *cis*-UCA (Hemelaar and Beijersbergen van Henegouwen, 1996). UCA therefore appears to be protecting against T^T-mediated immunosuppression at the same time that it is producing *cis*-UCA, which is an immunosuppressant, and also possibly inducing oxidative DNA damage, whose immunosuppressive role is unclear. It is probably no surprise that the action spectrum for the UV-induced systemic suppression of contact hypersensitivity in mice (de Fabo and Noonan, 1983) correlates with neither the action spectrum for *cis*-UCA production in mouse skin (Gibbs *et al.*, 1993) nor the action spectrum for T^T production. Thus, it is likely that a complex contribution of both chromophores leads to immunosuppression via a myriad of pathways. These multifarious effects of UCA on photoimmunosuppression are particularly important when its role in photocarcinogenesis is considered.

The derived action spectrum for nonmelanoma skin cancer in humans (SCUP-h) closely mirrors that for epidermal T^T formation. Barresi and coworkers (2011) clearly show that UCA protects against the production of T^T, so the presence of UCA would be expected to protect against photocarcinogenesis. However, several studies have shown no difference in cutaneous UCA content between those subjects with a past history of nonmelanoma skin cancer or cutaneous malignant melanoma and healthy controls.

Although the experiment comparing photocarcinogenesis in wild-type and histidinemic mice has not yet been reported, existing evidence demonstrates that increasing the *trans*-UCA content of mouse skin enhances photocarcinogenesis (Reeve *et al.*, 1989). This suggests that, although increased UCA may reduce T^T levels, the consequent reduction in tumor initiation and T^T-mediated photoimmunosuppression is not sufficient to counter the simultaneous increase in the level of *cis*-UCA, which is known to enhance photocarcinogenesis (Beissert *et al.*, 2001). The contributions of *cis*-UCA-induced intracellular ROS formation (Sreevidya *et al.*, 2010) and the recently reported ability of *cis*-UCA to induce the apoptosis of tumor cells through intracellular acidification (Laihia *et al.*, 2010) should be taken into account when attempting to unravel the very confusing overall contribution of UCA to the photocarcinogenic process.

Summary

The evidence presented by Barresi and colleagues (2011) suggests that *trans*-UCA acts as a natural sunscreen, giving lifelong, low-level protection against UVR-induced DNA damage and excessive keratinocyte apoptosis. However, *cis*-UCA, in addition to its well-reported immunosuppressive properties, may also initiate intracellular ROS production, oxidative DNA damage, and cell signaling that may negate any photoprotective effect (Figure 1). There is an obvious need to examine the relative susceptibility of wild-type and histidinemic mice to photoimmunosuppression and phototumorigenesis and also to assess the relevant photoresponses in histidinemic humans.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Lymphangiogenesis Linked to VEGF-C from Tumor-Associated Macrophages: Accomplices to Metastasis by Cutaneous Squamous Cell Carcinoma?

Martin S. Kluger[1] and Oscar R. Colegio[2]

During wound healing, dermal macrophages secrete lymphangiogenic vascular endothelial growth factor (VEGF)-C, and lymphatic vessels transport cytokines and cells to draining lymph nodes. In this issue, Moussai *et al.* show that macrophages in peritumoral nonlesional skin near squamous cell carcinoma secrete prolymphangiogenic VEGF-C. Their study suggests how tumor-associated macrophages and neolymphatic vessels may coordinate metastasis starting early in cutaneous squamous cell carcinoma.

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Roles for macrophage and lymphatic endothelial cells near squamous cell carcinoma

The primary functions of macrophages were first characterized in settings of classical inflammation in which they exert antimicrobial activity, serve as antigen-presenting cells required for the adaptive immune response, and promote healing by tissue remodeling at sites of injury. In contrast, tumor-associated macrophages (TAMs) are less inflammatory and contribute to tissue remodeling by promoting the proliferation and migration of endothelial cells (ECs) that lead to growth of new lymphatic vessels (lymphangiogenesis). The presence of TAMs generally correlates with a poor prognosis in most human cancers. Importantly, whereas lymphangiogenesis observed in many aggressive cancers correlates with metastasis to regional lymph nodes (e.g., in cutaneous melanoma and in head/neck and oral squamous cell carcinoma (SCC)), the presence and characterization of prolymphangiogenic TAMs in cutaneous SCC has not been previously described.

In this issue, Moussai *et al.* describe $CD68^+/CD163^+$ TAMs in peritumoral nonlesional skin (PTNL) of stage I cutaneous SCC as a source of vascular endothelial growth factor (VEGF)-C that correlates with an increase in lymphatic vessel density (LVD). These data showing lymphangiogenic VEGF-C produced by a defined subpopulation of TAMs raise a number of interesting questions about how macrophage-driven lymphangiogenesis may promote metastasis in SCC. Furthermore, these findings invite investigation of molecules expressed by lymphatic endothelial cells (LECs), or at recruitment of prolymphangiogenic TAMs, as potential therapeutic targets for preventing life-threatening metastases to regional draining lymph nodes.

Trophic macrophages are recruited by wounds and tumors

Macrophages are bone marrow–derived cells that initially circulate as monocytes and subsequently differentiate into tissue-resident macrophages. Resident macrophages support tissues by phagocytosing apoptotic cells, and they become trophic when activated, secreting growth, angiogenic, and lymphangiogenic factors needed for tissue remodeling. Wounding of tissues triggers an acute inflammatory response, characterized by the production of numerous cytokines and chemokines, which recruit and differentiate additional circulating monocytes into macrophages. Wound-associated macrophages have been proposed to coordinate new tissue formation and remodeling. More specifically, macrophages have been found to regulate vasculogenesis in wound healing. Whereas the specific

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